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The Morphology of Smoke Inhalation Injury in Sheep

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Pulmonary injury resulting from inhalation of chemical and particulate products of incomplete combustion is one of the principal determinants of mortality following burn injury. In this study, the histopathology of inhalation injury was examined in sheep. Mild, moderate, or severe smoke injury was produced in anesthetized sheep by insufflation with various doses of ambient temperature smoke, generated by burning polyethylene, wood pulp, and nonwoven cellulose pads. A total of 64 sheep were exposed and evaluated at times ranging from 15 minutes to 4 weeks after exposure. Morphologic changes in the lungs were studied using light microscopy and both transmission and scanning electron microscopy. The primary, dose-responsive injury observed was acute cell membrane damage in the trachea and bronchi leading to edema, progressive necrotic tracheobronchitis with pseudomembrane formation, and airway obstruction. These inflammatory and occlusive effects were followed by congestion, alveolar space edema, atelectasis, and bronchopneumonia. Morphologic changes occurring in the alveolar epithelium following high smoke dosage included intracellular edema in type-I cells, changes in the membrane-bound vacuoles of type-II cells, and septal thickening caused by interstitial edema. No capillary endothelial changes were observed.

Inhalation injury accompanies cutaneous burn injury in 32%–38% of severely burned patients, and the survival rate in patients with inhalation injuries is poor.[1] In recent years early diagnosis of smoke inhalation and evaluation of its severity have been of increased clinical interest, but more knowledge of the pathogenesis and effect of the injury would be beneficial to improve therapy.[2-9] Proper studies of inhalation injury, which is influenced by smoke temperature, the chemical and physical composition of the smoke, contact time, and the surface area exposed, require a reliable animal model.[5,8,10-14] The reproducible, dose-responsive sheep model used in this project has facilitated the study of the pathogenesis and the morphologic changes associated with smoke inhalation injury. Physiologic data collected from this study have been published elsewhere.[5]

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MATERIALS AND METHODS

The Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and the guidelines set forth in the *Guide for the Care and Use of Laboratory Animals*, National Institutes of Health Publication 86-23, were adhered to in this study.

Animals. Sixty-seven neutered, random source, 1- to 2-year-old male sheep weighing 24–46 kg were conditioned in covered outdoor runs, and fed commercial chow and water ad libitum during a 3-week period before experimental use. Baseline hematologic and blood chemistry data were accumulated before experimental use. Seven sheep were used as controls and 57 were exposed to smoke. The sheep individually received mild, moderate, or severe exposure to smoke and were studied at 15 minutes, 1 hour, 3 hours, 6 hours, 12 hours, 18 hours, 1 week, 2 weeks, or 4 weeks after smoke exposure. The majority of the sheep were studied 24 hours and 72 hours after smoke exposure. Before smoke insufflation, the sheep were fasted for 24 hours, intubated, anesthetized with methohexital sodium (Brevital Sodium, 9 mg/kg, Eli Lilly and Company, Indianapolis, IN) and paralyzed with pancuronium bromide (Pavulon, 0.03–0.04 mg/kg, Organon Pharmaceuticals, West Orange, NJ). The sheep were extubated after smoke insufflation. After smoke exposure, the sheep were housed in climate-controlled facilities at 74°–76°F (24°–25°C) and a relative humidity of 40% to 50%.

Smoke exposure. Smoke was generated by burning commercially available pads of polyethylene, wood pulp, and cellulose fabric in a 32-gallon (122-L) metal chamber.[5,12] The smoke contained 10%–14% oxygen, 3%–8% carbon dioxide, 0.7%–2.2% carbon monoxide, methane, ethylene, propylene,

acetaldehyde, and particulate combustion products, but no cyanide. Smoke exited from the combustion chamber to a volume-adjustable metal syringe, which permitted alternate insufflation of controlled volumes of smoke or atmospheric air. A standard unit of smoke required 50 seconds to administer and consisted of three successive insufflations of smoke with a tidal volume of 30 mL/kg and breath hold of 5 seconds followed by 10 successive ventilations with air. A mild smoke exposure consisted of 6 units, a moderate exposure 9 units, and a severe exposure 12 units. The smoke was equilibrated at ambient temperature to exclude all possibility of thermal injury to the airway.[5,8]

After smoke exposure, the sheep were extubated and allowed to breathe spontaneously in order to assess the natural progression of smoke inhalation injury.

Monitoring. Sheep studied at 15 minutes, 1 hour, and 3 hours were anesthetized with methohexital sodium only. In those sheep studied at all other times, anesthesia was induced with methohexital sodium (9 mg/kg) and maintained with alpha-chloralose (0.05 g/kg, Calbiochem, La Jolla, CA). The sheep were paralyzed with pancuronium bromide. After placement of catheters used for a concurrent physiologic study, the sheep were placed in a prone position and artificially ventilated. A volume-limited ventilator (Bear 2, Bear Medical Systems, Inc., Riverside, CA) with a tidal volume of 15 mL/kg was used at a respiratory rate of 12/minute. Sigh ventilation with a tidal volume of 21 mL/kg was applied every 3 minutes to prevent atelectasis. Lactated Ringer's solution was continuously infused at a rate of 1 mL/kg/hour.[5]

Pathology. Necropsies were performed on all sheep dying spontaneously or killed at the times noted above. A complete set of tissues was fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Special stains were used as needed. Tissue sample collection sites were the midtrachea, the tracheal bifurcation, right and left proximal and distal bronchi, apical and diaphragmatic lobes, and any other morphologically significant foci.

Specimens for transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were fixed in 2.5% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer solution at a pH of 7.3 at 4°C for at least 24 hours, then washed overnight in buffer. Specimens for TEM were post-osmicated for 1 hour in 2% osmium tetroxide, dehydrated in graded ethanols, and embedded in LX-112 resin. Thin sections were double stained with uranyl acetate and lead citrate. Specimens for SEM were dehydrated in graded ethanol-water solutions to absolute ethanol, then through graded ethyl alcohol-trichlorotrifluoroethane (Freon 113) solutions to absolute trichlorotrifluoroethane. The specimens were dried by the critical point method using monochlorotrifluoromethane (Freon 13). Dried specimens were coated in a DC sputter-coater with 20 nm of gold-palladium. The TEM and SEM specimens were examined in a Phillips 400T with STEM unit.

FIG. 1. (A) Light photomicrograph of a trachea taken 15 minutes after mild smoke exposure. Segments of intact epithelium (E) are adjacent to necrotic areas (N). The tracheal lumen contains sloughed epithelial cells, carbon particles (C), and proteinaceous debris. Original magnification: ×500. (B) Transmission electron micrograph of a similar area shows sloughed necrotic ciliated epithelial cells. Original magnification: ×7700.

RESULTS

The most significant lesion caused by smoke insufflation was necrosis and sloughing of respiratory tract epithelium. The necrosis was always most severe adjacent to the tip of the endotracheal tube and decreased in severity as the distance from the tip increased. Epithelial necrosis and sloughing were found in tissues collected as early as 15 minutes after smoke exposure (Fig. 1). Less significant smoke damage to the respiratory tract epithelium was characterized by clumping, swelling, and loss of cilia, blebbing, and surface erosion (Figs. 2, 3) as early as 1 hour after smoke exposure. There was evidence of increased mucus production by 12 hours (Fig. 4). Smoke damage was not uniform, but patchy; it was possible to find foci of apparently unaffected epithelium within areas of severe damage. These foci were usually in folds and crypts and were more common in sheep after mild smoke exposure (Fig. 4).

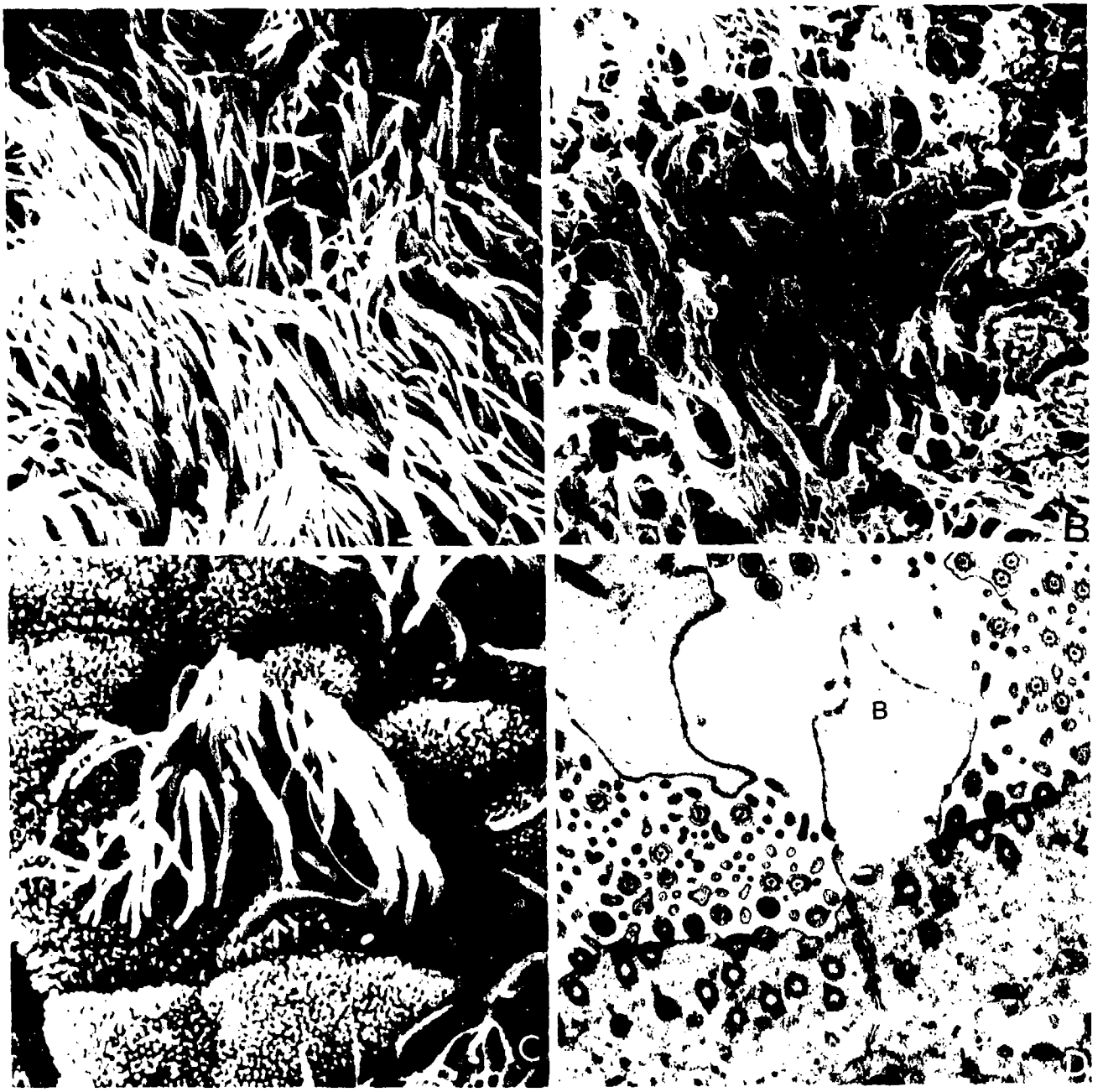

FIG. 2. (A) Scanning electron micrograph of trachea from a control sheep shows normal complement of cilia. (B) Twenty-four hours after exposure to mild smoke there is matting and disorientation of the few remaining cilia. (C) Blebbing of the surface membrane (arrow). (D) Transmission electron micrograph of area similar to Fig. 2C showing surface bleb (B). Original magnification: ×6400.

FIG. 3. Scanning electron micrograph of a trachea 24 hours after moderate smoke exposure. No cilia are present and there is erosion of some cell surface membranes (arrows). Original magnification: ×3200.

FIG. 4. Light photomicrograph of a trachea 12 hours after mild smoke exposure showed damage to surface epithelium, abundant mucus-producing cells (arrows), and a crypt (C) containing well-preserved epithelium. Original magnification: ×500.

In this study the extent of injury was directly related to the dose of smoke and the time elapsed after contact with the smoke. With mild smoke exposure, there were mild inflammatory changes and superficial erosion of respiratory epithelial cells. With severe smoke exposure, there were extensive inflammatory changes and erosion extending down to and in some cases including the basal epithelium, leaving an ulcerated surface (Fig. 5).

The necrotizing injury was associated with an inflammatory response that started as early as 2 hours after injury and continued until death or resolution of the injury. This inflammatory response was manifested grossly by the formation of pseudomembranes (fibrinonecrotic tracheobronchitis) and sloughing of the respiratory epithelium (Fig. 6). Membrane formation was typically seen in major airways and became progressively thicker with higher doses of smoke and time after exposure (Fig. 7). Edema and neutrophils were seen as early as 2 hours in the trachea and bronchi. The neutrophils were present in the lamina propria, epithelium, and airway lumens. The acute inflammatory cell response was maximal by 24 hours. Bacterial colonization and infection were microscopically evident at 72 hours after severe smoke exposure (Fig. 7). Carbon particles were present in the trachea and terminal airways in the first few hours after exposure but were rarely seen later in the disease process. They were not seen at any time in alveolar spaces.

The respiratory tract epithelium generally became metaplastic if the basal cell layer survived (Fig. 8). The metaplastic change was present as early as 12 hours after mild smoke exposure. There was complete repair of the respiratory epithelium in the sheep that underwent mild smoke exposure with return of normal cilia populations within 2 weeks of smoke injury. Complete repair required 4 weeks in the sheep surviving moderate smoke exposure.

Parenchymal changes were generally in the anterior and dependent areas of the lungs. Atelectasis was present concomitantly with early occlusion of terminal airways and was evident both grossly and microscopically (Figs. 9, 10). The debris occluding an airway was frequently associated with areas of inflammatory infiltrate, edema, hyperemia, congestion, and atelectasis (Fig. 11). Bronchopneumonia was a prominent feature in these sheep and was characterized by the collection of neutrophils in airways and alveolar spaces (Fig. 12). The lung parenchyma in dorsal areas often had minimal atelectasis and congestion, but pneumonia was rare. Perivascular, interlobular, and septal edema was progressive and severe in some sheep with marked dilatation of the lymphatics, blood vessels, and septae. Marked alveolar edema usually occurred after 24 hours.

Smoke-induced alveolar epithelial damage was not seen with light microscopy but was identified ultrastructurally in the sheep that underwent severe smoke exposure. The epithelial changes included mild edema in type-I cells and changes in the membrane-bound vacuoles in type-II cells. Additionally there was mild interstitial edema with septal thickening (Fig. 13). Vascular endothelial injury was not seen.

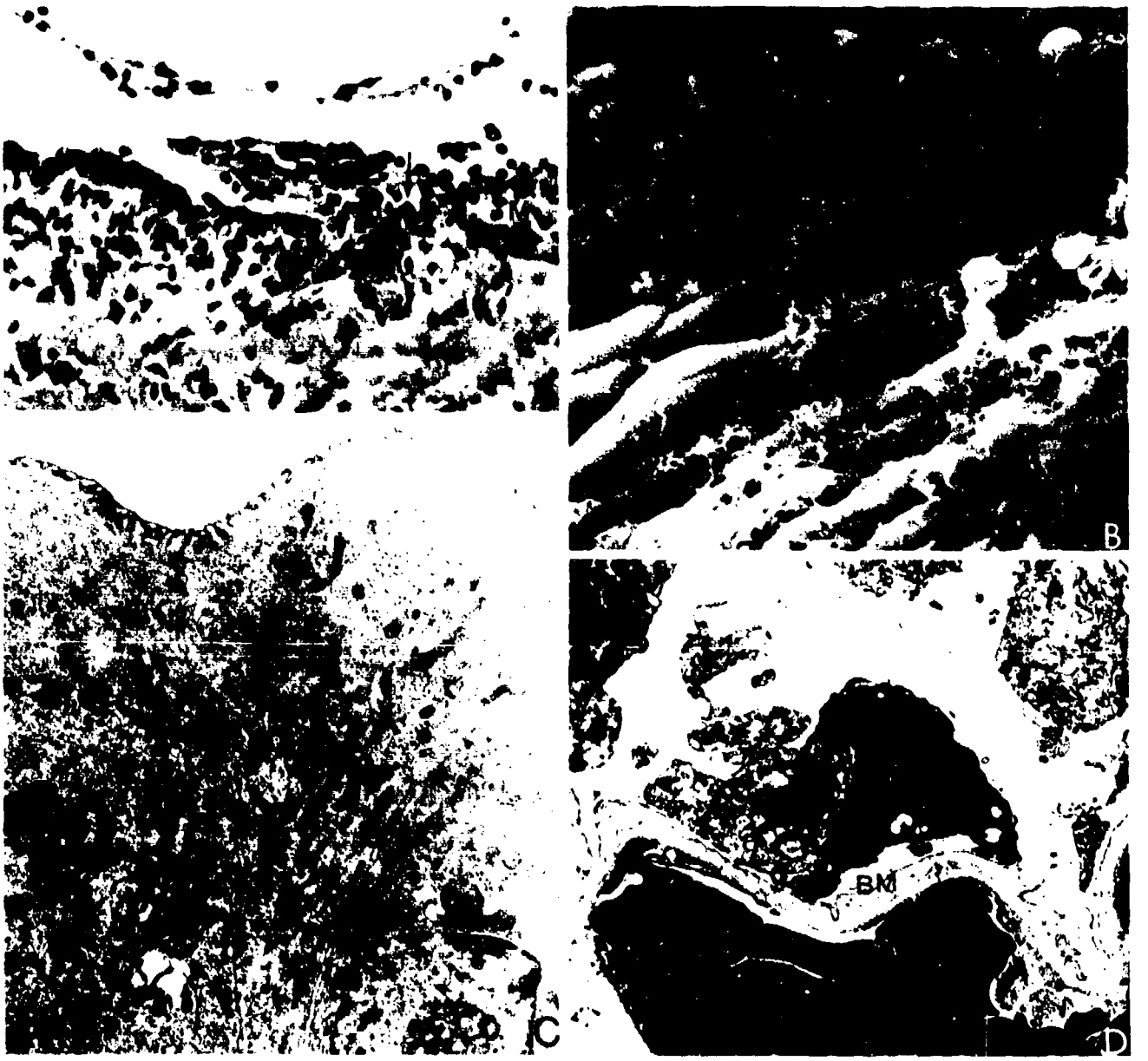

FIG. 5. (A) Light photomicrograph of a trachea 12 hours after mild smoke exposure shows pseudomembrane (M), ulceration of respiratory epithelium (arrow), and early epithelial metaplasia (arrowhead). Original magnification: ×325. (B) Scanning electron micrograph of a similar area to Fig. 5A shows metaplastic epithelium partially covered by pseudomembrane and red blood cells. Original magnification: ×1600. (C) Transmission electron micrograph of metaplastic epithelium. Original magnification: ×6000. (D) Transmission electron micrograph of ulcerated epithelium. Cells have sloughed down to the basement membrane (BM). Original magnification: ×2800.

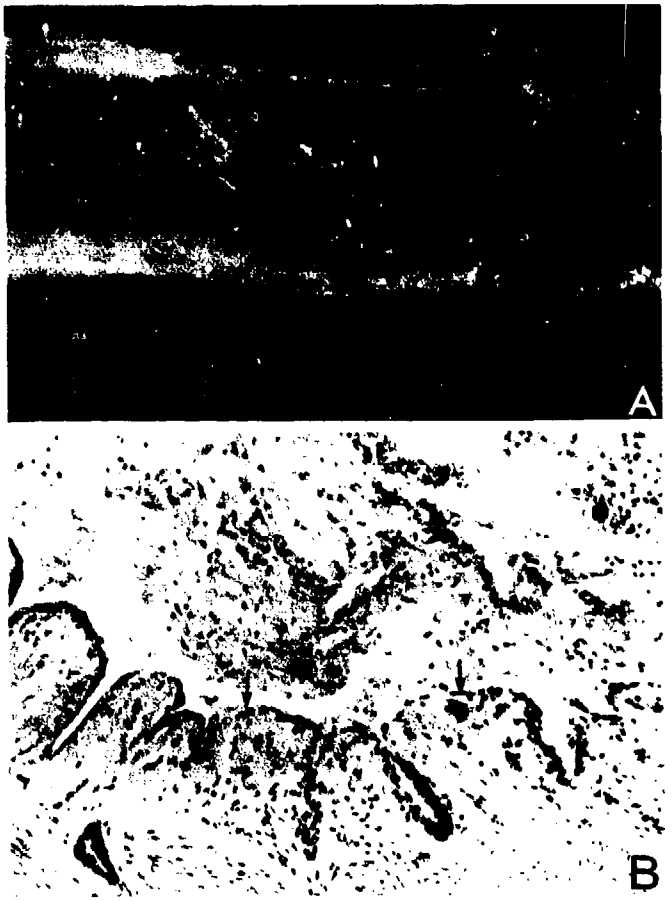

FIG. 6. (A) Gross photograph of the trachea of a sheep 24 hours after moderate smoke exposure shows a pseudomembrane (M) that partially occludes the airway. (B) Light photomicrograph of a bronchus containing extensive pseudomembrane formation (M) and erosion of respiratory epithelium (arrows) 12 hours after mild smoke exposure. Original magnification: ×325.

FIG. 7. Light photomicrograph of a trachea 72 hours after severe smoke exposure shows diffuse severe inflammatory response (I) and bacterial colonization (B) of the necrotic epithelium. Original magnification: ×325.

FIG. 8. (A) Light photomicrograph of tracheal epithelium 72 hours after mild smoke exposure shows squamous metaplasia and associated inflammatory response. Original magnification: ×500. (B) Transmission electron micrograph from same area as in Fig. 8A shows metaplastic epithelial cells with microvilli (MV) on the luminal surface. Intercellular bridges (arrows) are present in deeper cell layers. Original magnification: ×3000.

FIG. 9. Gross photograph of a lung 24 hours after moderate smoke exposure illustrates typical atelectic change associated with airway occlusion. The atelectasis is most severe and common in anterior and dependent areas of the lung.

FIG. 10. Light photomicrograph of lung 12 hours after moderate smoke exposure. A pseudomembrane partially occludes a bronchus (M) and there is associated atelectasis (A) and edema (E) adjacent to essentially normal lung (N). Original magnification: ×125.

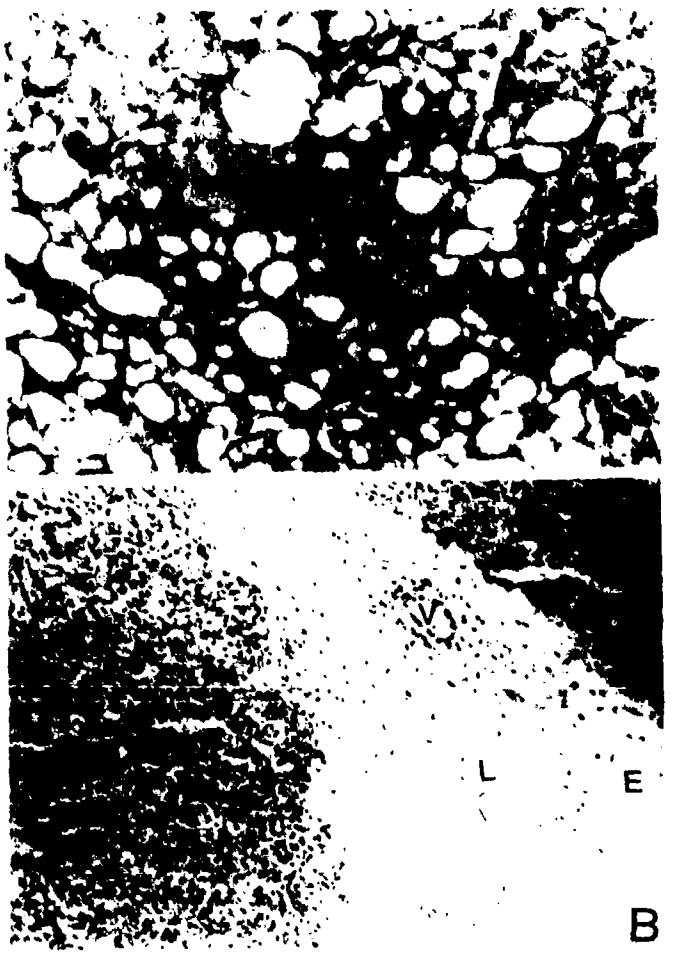

FIG. 11. (A) Light photomicrograph of lung 12 hours after mild smoke exposure illustrates early periarteriolar edema (P) and congestion of alveolar septae (S). Original magnification: ×325. (B) Photomicrograph of lung 24 hours after mild smoke exposure shows marked dilatation of an interlobular lymphatic (L) and blood vessels (V) and edema (E). Note adjacent septal congestion and atelectasis (A). Original magnification: ×325.

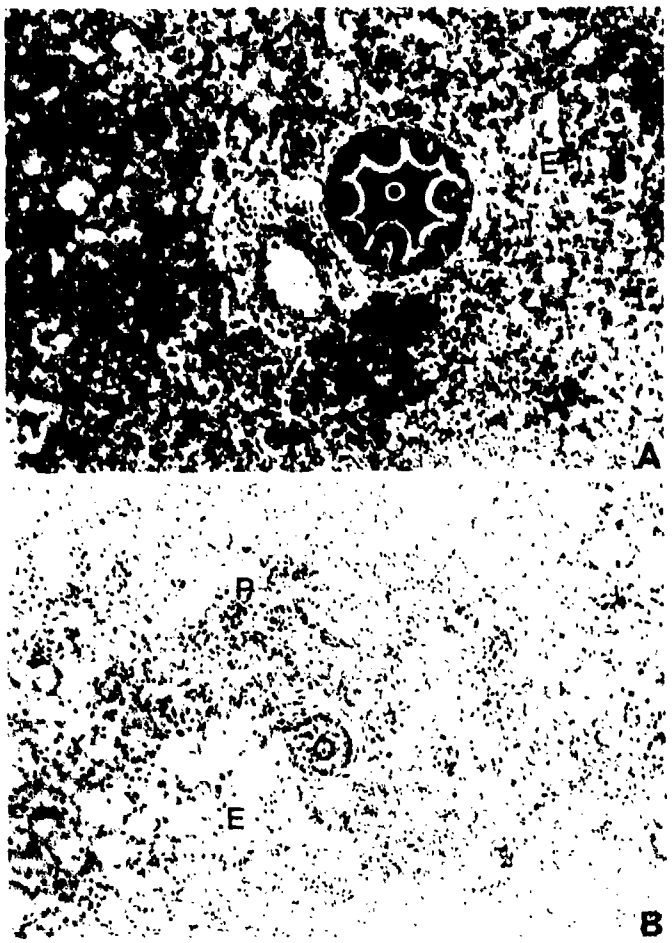

FIG. 12. Light photomicrographs of lung (A) 24 hours after moderate smoke exposure and (B) 72 hours after severe smoke exposure. Both show airway occlusion (O), pneumonia (P), and severe alveolar edema (E). Original magnification: ×375.

In the sheep allowed to live past 72 hours, resolution of the bronchopneumonia generally was uneventful in both the low- and moderate-exposure sheep (Fig. 14). In the high-dosage sheep that died, death always occurred before 72 hours as a result of severe airway obstruction and resultant hypoxia.[5]

DISCUSSION

The severity of inhalation injury in a spontaneously breathing animal is influenced by hyperventilation in response to the carbon monoxide content of the smoke. In those animals with elongated nasal passages, epiglottic closure or early bronchospasm may cause further variability in severity of injury.[5] Smoke exposure in this study was done under general anesthesia using endotracheal intubation to avoid the variations in the depth of breathing that influence contact area. Variability of combustion, with respect to smoke composition, was minimized by use of a relatively large combustion chamber.

The extent and severity of the injury to the epithelium was directly related to the smoke exposure. The histologic finding of tracheal epithelial metaplasia, which occurs following erosion and ulceration, suggests that mild and sometimes moderate damage from smoke exposure is quickly repaired. Histologic changes in sheep that underwent severe smoke exposure showed further deterioration of airway epithelium, and congestion and atelectasis of the lung were more marked by 72 hours after exposure. The parenchymal changes were most distinct in dependent areas of the lung.

Morphologic changes in the alveolar epithelial type-I and type-II cells were seen by electron microscopy only after severe smoke exposure. The ultrastructural changes in the type-II cells raise the possibility of concomitant changes in the normal complement of pulmonary surfactant, which have been reported to be associated with smoke inhalation.[15] These ultrastructural changes were usually present along with alveolar and interstitial edema; however, vascular endothelial changes were not recognized in any of the sheep. It is possible that microvascular permeability increased without morphologic change. Some authors have attributed the progressive pulmonary edema observed in previous studies of inhalation injury to alveolar epithelium damage and pulmonary macrophage-induced or neutrophil-induced increases in pulmonary microvascular permeability.[1,10,16-18] These alterations may be the result of hypoxia associated with airway obstruction rather than a direct toxic effect of smoke on the alveolus.

The results of this study suggest that the cellular changes evoked by smoke inhalation are related to the contact effects of chemicals or radicals in the smoke, which exert detrimental effects on cell membranes, resulting in cell death.[19,20] The damage to the respiratory epithelium was seen in both major and minor airways but the severity of injury was related to the amount of smoke and the elapsed time after contact with the smoke.

The edema, congestion, atelectasis, and pneumonia in this model appear to be the result of occlusion of airways by desquamated necrotic endobronchial tissue. Accumulation of occlusive exudate in dependent airways is probably related both to the effect of gravity and to the loss of effective normal elimination of exudate by ciliary action and coughing.[21-23] The injury also triggers an inflammatory response following moderate to severe smoke exposure that contributes to occlusion of the airways and, eventually, the alveolar spaces. Activation of the inflammatory cascade may be a contributing factor.[24] Occlusion of the airways is associated with hypoxia and, in later stages, pneumonia. This pathogenetic sequence is consistent with prior studies.[16,25-28] Shimazu found that moderate inhalation injury was associated with pseudomembrane formation in the major airways and a mortality rate of 30% by 72 hours. Severe inhalation injury caused 10%–14% mortality by 24 hours and

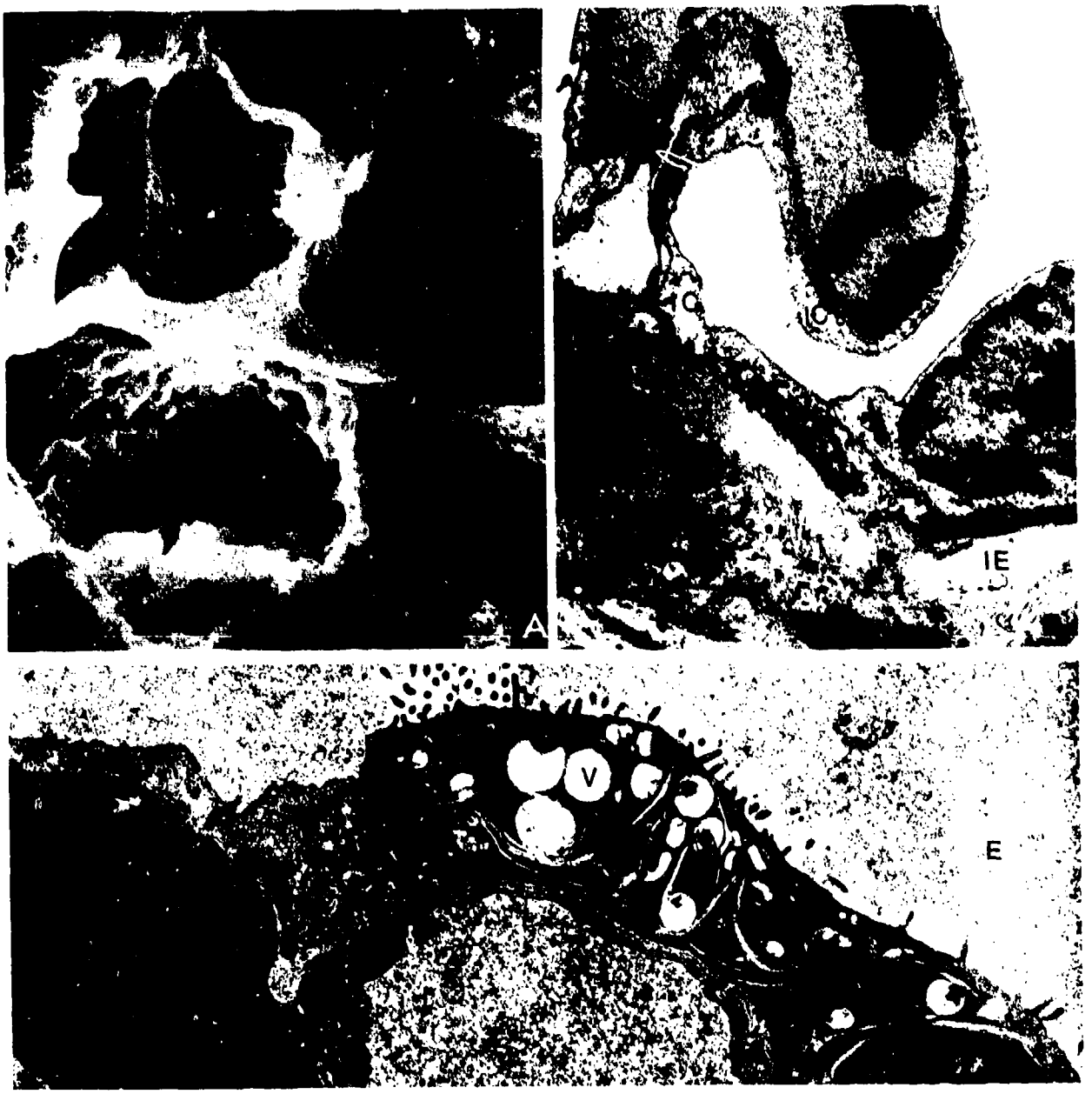

FIG. 13. (A) Scanning electron micrograph of lung 24 hours after severe smoke exposure shows thickening of the interstitial areas caused by edema as well as swelling of the epithelial cells. Original magnification: ×800. (B) Transmission electron micrograph shows edema in type-I cells (IC) and interstitial edema (IE). Original magnification: ×6000. (C) Transmission electron micrograph of lung 24 hours after severe smoke exposure shows alveolar edema (E) and empty vacuoles (V) in a type-II cell. Original magnification: ×3000.

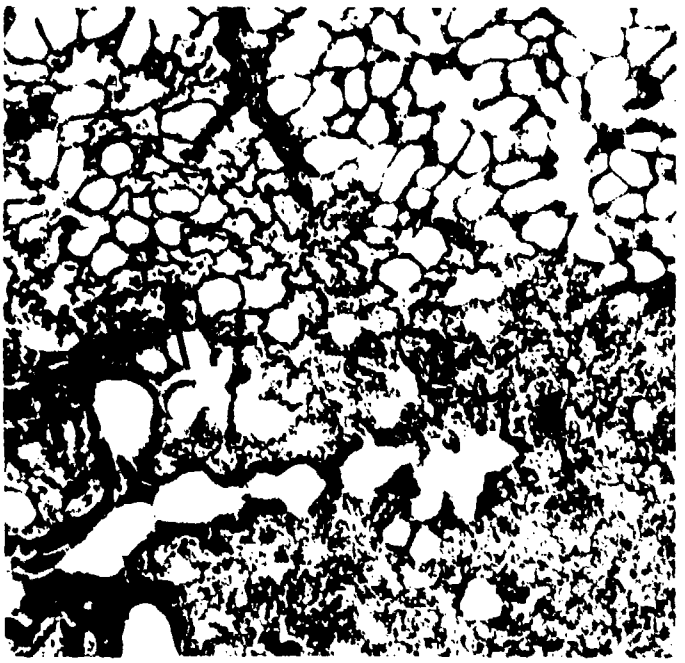

FIG. 14. Light photomicrograph of lung 1 week after moderate smoke exposure illustrates the clearing of alveolar spaces by macrophages (arrows). Original magnification: ×125.

30%–100% mortality by 72 hours, and a thick pseudomembrane always formed in the airways. Total loss of airway epithelium was associated with 100% mortality.

This study suggests that control of the inflammatory process may limit the airway obstruction caused by smoke inhalation. If the toxic injury and resulting inflammatory response can be controlled and pneumonia prevented, repair of the respiratory tract should be unimpeded and the mortality rate from inhalation injury would be reduced.

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